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Development of a processed cereal food fortified with iodine

Desenvolvimento de um produto cerealífero processado fortificado em iodo

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Abstract

Deficient or excessive iodine dietary intake can result in serious pathology problems, mostly in relation with thyroid gland metabolism which may represent a direct threat to the human being. Therefore it is important that the intake of this mineral is adequate according to the recommended daily intake. It was the aim of this study to develop a processed cereal food fortified with iodine such as a prophylactic measure to combat iodine deficiency without using iodized salt.

A total of four recipes of sweet biscuits were formulated. Different recipes contain increasing levels of potassium iodide, to be precise 0.5, 0.75 and 1mg per 100g flour and one of them didn't have any addition of potassium iodide.

The iodine was determined by an ion-pair reversed-phase HPLC with ultraviolet detection, a simple, practical and sensitive method. Sample preparation was done by alkaline dry ashing, and therefore a mean extraction recovery of iodine of 77.1% was obtained.

The iodine concentration found in the sweet biscuits was substantially lower than the iodine concentration added. The mean recovery of iodine at the three different recipes was 6.3%, 7.7% and 7.9%, respectively.

Although, it is technologically possible to do iodine fortified processed cereal food without compromising the texture, the flavour and the acceptability, a high loss of added iodide was verified. However, quantification method of iodine need more studies.

Key words

Iodine intake, fortification, processed cereal food, iodine extraction, ion-pair RP-HPLC

Resumo

Uma ingestão deficiente ou excessiva de iodo pode resultar em sérios problemas patológicos, maioritariamente relacionados com o metabolismo da glândula tiróide, o que pode representar uma ameaça direta para o Homem. Assim, é importante que a ingestão deste mineral seja adequada, de acordo com a ingestão diária recomendada. Deste modo, foi objetivo deste estudo desenvolver um produto cerealífero processado fortificado com iodo, sem recorrer à utilização de sal iodado, como medida profilática de combate à deficiência em iodo.

Foram formuladas quatro receitas de bolachas na totalidade. As diferentes receitas continham um nível crescente de iodeto de potássio nomeadamente, 0,5; 0,75 e 1mg por 100g de farinha, enquanto a uma destas não foi adicionado iodeto de potássio.

O iodo foi determinado por cromatografia líquida de elevada *performance* com par iónico em fase reversa e deteção em ultravioleta, um método simples, prático e sensível. A preparação da amostra foi realizada através de um método de cinzas em meio alcalino, obtendo-se uma recuperação de extração média de 77,1%.

A concentração de iodo encontrada nas bolachas foi substancialmente menor do que a concentração de iodo adicionado, visto que a sua recuperação média nas três receitas distintas foi de 6,3%, 7,7% e 7,9%, respetivamente.

Embora, tecnologicamente, seja possível produzir um produto cerealífero processado fortificado em iodo, sem comprometer a textura, o sabor e a aceitabilidade, verificou-se que existem elevadas perdas do iodo adicionado. No entanto, o método de quantificação de iodo precisa de mais estudos.

Palavras chave

Iodo, fortificação, produto cerealífero processado, extração de iodo, HPLC em fase reversa com par iónico.

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Abbreviations

AAS - Atomic absorption spectrometry

HPLC - High-performance liquid chromatography

i.d. - Internal diameter

KI - Potassium iodide

min – minutes

NAA - Neutron Activation Analysis

T3 – Triiodothyronine

T4 – Thyroxine

UV – Ultraviolet

Introduction

Minerals, which generally are combined in food in form of minerals salt, are an important component of human body, representing 4% to 5% of its composition[1].

These nutrients establish balance among them and the organic composts, therefore its excess, or lack of it, may affect human health. The required amount of each mineral is not proportional to its physiological importance. Through a healthy diet, minerals are acquired in enough quantity and balance[1].

Among these minerals there is iodine, one of the most important tracing elements for animals, including human beings, especially in their early stages of life. However, for plants it is considered a nonessential mineral[2]. Iodine is ubiquitous in the biosphere and it is able to change between solid, liquid and gaseous phases[2, 3], besides it readily sublimes at room temperature[3].

This mineral's biogeochemical cycle is complex and involves processes from ocean release to soil and vegetation absorption[2].

We can find 20 to 30 mg of iodine in human body, 75% of which are in the thyroid gland[1]. It is the key element for the synthesis of thyroid hormones, triiodothyronine (T3) and thyroxine (T4), which play a outstanding role in the metabolism of most cells of the organism, in the process of early growth and in the development of most organs, especially brain, particularly during fetal development and childhood [2, 4-6].

Deficient or excessive iodine dietary intake can result in serious pathology problems, mostly in relation with thyroid gland metabolism which may represent a direct threat to human beings [2, 5, 7, 8].

Iodine deficiency disorder is a significant worldwide public health problem, especially in developing countries[9, 10]. Studies released in Portugal have shown

an inadequate iodine supply in pregnant women[4]. In opposition, there are a substantial number of countries where the level of iodine intake is too high[9].

Iodine deficiency is associated with many abnormalities like stillbirth, miscarriages and neurocognitive problems, namely mental retardation, dwarfism and hearing loss. But a high iodine intake can cause hyperthyroidism and allergies. On the other hand, an uncontrolled increase in iodine intake may also cause diseases, like hypothyroidism that can lead to endemic goiter (a swelling of the butterfly shaped thyroid gland in the neck) in adults or creatinism in children (congenital hypothyroidism resulting in physical and mental stunting during prenatal development) [2, 4, 5, 9, 10].

Hence, it is important that the recommended daily intake of iodine is known. The World Health Organization (WHO) recommends a daily intake of 150-200 µg of iodine. However in pregnancy and lactation the needs are quite increased (220 µg and 290 µg of iodine respectively), since a mild to moderate iodine intake in these stages may have deleterious consequences in health[4, 5, 11].

The major source of iodine for man is food chain[2]. While iodine abundance in our Earth´s crust is only 0,000061%[8], oceans are the main reservoirs of this element. Iodine content in sea water are approximately 50 mg/t and in organisms such as algae, seaweed and sea sponges are 19 g/kg dry weight[8], which explain why marine fish and seaweed are the richest natural food sources of iodine. The seaweed iodine concentration is 100 – 1000 times higher than in fish. For example, fish with very high iodine concentration like sardine and horse mackerel have approximately 250µg of iodine/100g, while *kombu,* a typical and commonly seaweed, contains approximately 130.000µg of iodine/100g[5]. So, there are few Japanese patients with iodine deficiency[12]. Milk, dairy products and chicken eggs

are also rich in iodine due to iodized animal feed[13]. Another point of view considering fruit and vegetables have rather low iodine contents with exception of herbs, dark and leafy vegetables [14]. Although water and beverages can contain iodine they would not be a useful dietary source of this mineral [14, 15]. Some pharmaceuticals and artificial isotopes also contain iodine which are used in medical, biological and biochemical researches [8]. There are some products, like baked products with high iodine content, that come mainly from the addition of iodized salt [14].

Iodine variability needs to be taken into consideration since it explains why the same food item may have widely different iodine content depending on the locality where it was produced. Costal areas' soils contain substantial amount of iodine in comparison with inland soils. Therefore, plants which grow in iodine-rich soil will have high amount of this element [14]. Furthermore, we can find unplanned alterations in the iodine intake due to changes in farming practice or iodine-containing chemicals in the food industry [2]. Studies have claimed that iodine may occur in milk as a result on the use of iodophores, as cow teat sterilizers and equipment sanitizers [16]. Changes in the modern diet may have decreased the reliability of iodine as a replacement for resource availability [2].

Although the reduction of salt intake is a worldwide public health measure, salt has been chosen as a vehicle for iodine fortification in many countries to compensate the iodine deficiency [4, 17]. Studies suggested that a decreased use of iodised salt may be a possible reason for the decreasing of iodine status [18]. In most of the countries around the world sodium intakes are more than they need, approximately 9 to 12 g/day[19]. An excessive amount of sodium intake is mostly associated with high blood pressure and increase the risk of cardiovascular

disease, which is one of the causes of mortality from stroke in Europe [20-22]. To get all your iodine from salt, you would need more than the daily allotment of sodium recommended (1,5g sodium/day) [23].

The inhomogeneity of iodine in iodized salt make salt a non good predictor for iodine intake [24]. Moreover, iodine salt may be volatile and lost through cooking model conditions[17, 24]. For the mentioned reasons it makes more sense to get iodine from food.

There is a data of iodine fortification in food products, but through iodised salt, such as the national bread fortification program from Food Standards of Australia and New Zealand (FSANZ)[15]. However, evidence from Tasmania and Gippsland suggested that bread iodine fortification alone was unsuccessful to reduce iodine deficiency in the pregnant, breast-feeding women and their young babies [25, 26].

To find a prophylactic measure to combat iodine deficiency, that not iodised salt, is a very important challenge. Processed cereal foods are possible food vehicle for iodine fortification[24], once as commonly consumption for Portuguese population.

Commonly researches have been using potassium iodide (KI) for fortification due to its higher iodine availability and lower cost[17]. However, the high level of potassium from KI causes off-flavours, such as metallic or bitter taste. Thus, it is important that these studies look for the right percentages of potassium iodide to develop processed cereal food with texture, flavour and colour according to the consumer preferences.

Although the iodine quantification method is important, the sample preparation is essential too, since to convert iodine into a certain form prior to the final determination, the samples need to be dissolved completely which require high temperatures, strongly oxidising and reducing reactions.

The most [13] used sample pretreatment methods are microwave-assisted pressure digestion[27]; Schoniger combustion[7]; alkaline dry ashing[13, 28-30] (preferred procedure due to its non-existing acid solutions) and solubilization with tetramethylammonium hydroxide[2, 7].

There are many methods for iodine determination like spectrophotometric method[27] based on Sandell and Kolthoff reaction[29, 31], chromatography (High-performance liquid chromatography (HPLC)[28, 32], chromatography gas), spectral methods (Inductively coupled plasma - optical emission spectrometer, Inductively coupled plasma – mass spectrometry (ICP-MS)[31, 33]), neutron activation analysis (NAA)[31], electrochemical methods and a combination of these methods[7, 31]. Iodine is rarely determined by atomic absorption spectrometry (AAS), but indirect AAS can be successfully applied[13, 27, 34]. Though, the spectrophotometric method is very sensitive and has a very good limit of detection, ICP-MS is the most important of all the iodine quantification methods and more frequently used because it is simple, quick and does not need toxic reagents such as arsenic oxide, brucine, among others, but it has high costs involved[7]. On the one hand, the NAA reached accuracy and sensitivity, although it was not suitable for routine practice. On the other hand, HPLC, particularly ion-pair reversed-phase HPLC with ultraviolet (UV) detection, is a simple and widely method to determine the total iodine in biological material[32]. According to Lijun Cui *et al*[32], this procedure have suitable sensitivity and is more practical than the mentioned preceding methods which meet the preestablished aims for this study.

Objectives

The aims of this study were: i) to select the most adequate processed cereal food for iodine fortification; ii) to develop iodine fortified processed cereal food; iii) to investigate the effects and impact of iodine fortification in flavour; iv) to determine a total iodine in this processed cereal food after cooking by ion-pair reversed-phase HPLC with UV detection.

Materials and methods

Process of a processed cereal food

Sampling **-** The aims of this study were applied in bread and in sweet biscuits. The bread was produced in an experimental laboratory CERES in Porto. A commercial wheat flour type 65 was used. The yeast was obtained from FALA AZUL, Portugal by multiplication of cells from a pure culture of yeast species *Saccharomyces cerevisiae* specially selected. The dough improver used was Cerpan Classic, Porto. The sweet biscuits were produced in an experimental laboratory FCNAUP in Porto. A commercial wheat flour type 65, Skimmed butter (Primor ®), skimmed milk (Agros ®), sugar (RAR ®) and egg yolk were used. Potassium iodide was employed for fortification in both cases.

Substitution recipes- A total of 8 recipes, 4 of bread and 4 of sweet biscuits were formulated. Recipes A, B and C of bread contain different proportions of NaCl (0.6, 1 and 1.4 g per 100g flour weight basis, respectively) and the same amount of KI (160µg per 100g flour weight basis). Moreover, control containing only KI (160µg per 100g flour weight basis) and the basis ingredients. For sweet biscuits, recipes

A1, B1 and C1 contain increasing levels of KI (500; 750 and 1000 µg per 100g flour, respectively). The control 1 only contains base ingredients.

Bread and sweet biscuits development- All breads were prepared in pilot laboratory homemade bread machines. The control wheat bread formulation included 500g wheat flour, 300ml water (vol/wt, flour basis), 10g dry yeat (wt/wt, flour basis), 5g dought improver (wt/wt flour basis) and 160µg potassium iodide (wt/wt flour basis). The remaining bread samples were made with the same formulation and the partial addition of salt at different levels, according to the recipes shown in Table 1. The wheat bread process consisted in dissolving potassium iodide and salt in 60% water. All ingredients were automatically mixed, kneaded, fermentated and baked during 3hours and 20 minutes in homemade bread machines. Each recipe provides approximately 19 balls into approximate 40g each.

The sweet biscuits were handmade baked in a Gastrotecnia kitchen oven. The control sweet biscuits formulation included 500g flour, 100g sugar, 100g butter, 125ml milk and 4 yolk eggs. The remaining sweet biscuits samples were made with the same formulation and the partial addition of potassium iodide at different levels according to the recipes shown in Table 1.

The sweet biscuits process consisted of mixing the flour, butter, sugar and yolk eggs with milk. In an earlier stage potassium iodide was dissolved into the milk. The ingredients were incorporated in the bowl and manually kneaded for 15 minutes. Then dough was moulded into approximate 100 sweet biscuits and baked in an oven at 180º for 15 minutes.

Table 1 - Potassium iodide and NaCl levels in different bread and sweet biscuits recipes

Recipes	***Bread***				***Sweet biscuits***			
	Control	**A**	**B**	**C**	**Control 1**	**A1**	**B1**	**C1**
KI level (µg/100g flour)	160	160	160	160	0	500	750	1000
NaCl level (g/100g flour)	0	0.6	1.0	1.4	-	-	-	-

Sensory analysis

Descriptive analysis was conducted by untrained panel (7 members) to evaluate the intensity of sensory characteristics of cereal food fortified with iodine.

Iodine Quantification

Iodine was determined only in sweet biscuits.

Chemicals and Reagents- The following reagents and chemicals were used: sodium dihydrogen phosphate monohydrate ($NaH_2PO_4.H_2O$), Germany; disodium hydrogen phosphate dehydrate ($Na_2HPO_4.2H_2O$), Germany; methanol, gradient HLPC grade, Spain; octylamine ($C_8H_{19}N$), 99,5% purity, Switzerland; sulfuric acid (H_2O_4S) (95-97%), Germany; sodium nitrate ($NaNO_3$), 99,0% purity, Japan; potassium iodide (KI), United Kingdom; potassium hydroxide (KOH), Germany; potassium chloride (KCl), 99% purity, Spain; nitric acid (HNO_3), Germany; hydrochloric acid (HCl), 32%, Germany; ammonium persulfate ($(NH_4)_2S_2O_8$), 98% purity, Germany and ultra pure water.

*Preparation of Standard Solutions/ iodine calibrators***-** The standard stock solution of iodide (1000mg $L^{-1)}$ was prepared by dissolving KI (1038 mg) in ultra pure water. Using a intermediate solution of 100mg L^{-1}, working standard solutions at 0.05,

0.1, 0.2, 0.5, 1, 2 and 5 mg mL^{-1} in ultra pure water were prepared, immediately before use.

Sample preparation - Firstly, the larger pieces of sample are cut into small portions and homogenized. Five different extraction methods were used: two different acid digestions (one with nitric acid and another with a mixture of sulfuric acid and chloride acid)[2]; a standard digestion method using ammonium persulfate[35, 36]; a chemical extraction using potassium chloride (KCl)[2] and a alkaline dry ashing[29, 30, 37]. The alkaline digestion consisted in accurately weight approximately 0,5g sample, add 1ml of 2M potassium hydroxide solution and 1ml of ultra pure water and dry at 105ºC for 20h. After that, place in a cold muffle furnace heat at 150ºC for 30 min, then raise the temperature to 600ºC and maintain for 1h. Dissolve the obtained ash in 9ml of ultra pure water and 1ml of 1M hydrochloric acid using an ultrasonic bath (5 min). Centrifuge the mixture at 5000 rpm for 15min[29]. The extractions were made twice.

Recovery trials - To evaluate the alkaline dry ashing performance, a recovery trial was made in which a standard solution 1000 $\mu g\ L^{-1}$ and 2000 $\mu g\ L^{-1}$ was added to the sweet biscuit sample control (without iodine addition).

Chromatographic System and Conditions- Iodine was determined on an isocratic reversed-phase HPLC method (Jasco HPLC system, consisting of a Rheodyne 7125 sample, one Jasco PU-2089 Plus pump and a Jasco UV-970 PDA detector) using UV-vis detector set at 250nm. The mobile phase consisted of a mixture of water phase (containing 5mmol L^{-1} octylamine, 5 mmol L^{-1} sodium dihydrogen

phosphate and 5 mmol L^{-1} disodium hydrogen phosphate, pH adjusted to 6.0 with sulfuric acid) and methanol in the ratio 80:20 (v/v) at a flow rate of 0,900 mL min^{-1}. The chromatographic separation was performed on a Diamonsil C_{18} column (250 x 4.6mm i.d., 5 µg; ACE, 5C18), which temperature was maintained constantly at 40ºC using a thermostatically controlled column oven. The chromatographic run time for each analysis was 10 min [32]. Injection content was 20µl. For each sample triplicate analysis were made.

Statistical analysis

Statistical treatment of the data was performed using Microsoft Office Excel v2007.

Results and discussion

Selection the processed cereal food fortified with iodine

The bread is a staple food in the human diet in many countries [38] and a cereal product with the best retention of iodine[24]. But the chance to fortify the bread with iodine requires a more detailed work, since its daily consumption is changeable, making not the most effective strategy to control iodine status. EPIPorto, a epidemiological study carried out in Portuguese adult population, showed a bread diary consumption of 124,8g, which was lower in women (113,6g/d, dv 61,1) than in men (136,0g/d; dv 73,6) [39]. So, in an earlier stage, to fortify sweet biscuits with the recommended portion facilitated this study.

Sensory analysis

After the processing of the sweet biscuits, they were analyzed sensorially. All elements of the panel considered that appearance, odour attributes and textures

of the sweet biscuits from all recipes (Control, A1, B1 and C1) were normal and pleasant. Considering aroma, sweet biscuits from the recipe C1, with the highest level of fortification, were considered, adstringent by two members.

Iodine Quantification

Most analytical techniques require sample decomposition, which is a delicate problem in iodine determination[2].

Conserning applying acid digestions, it can be seen that nitric acid has a similar retention time to iodine which doesn't allow to determine iodine. Besides, the sulfuric and chloride acids mixture are not able to a complete digest the sample. Therefore it was not possible to achieve results to this method. According to Xiaolin Hou et al in acid digestions methods may occur volatile species of iodine, like I_2 or HI, which can easily be evaporated from acidic solution by heating, leading to iodine loss[37]. On the other hand, digestion method using ammonium persulfate and chemical extraction method using potassium chloride, do not allow the detection of iodine by HPLC because they do not remove interfering substances. In this case this extraction was not applied.

Considering all the used pretreatment sample methods, alkaline dry ashing was the most suitable one, because it allowed a full digestion of the sample and removed the interfered substances which permitted a clear determination of iodine which can be seen in the chromatogram (Figure 1).

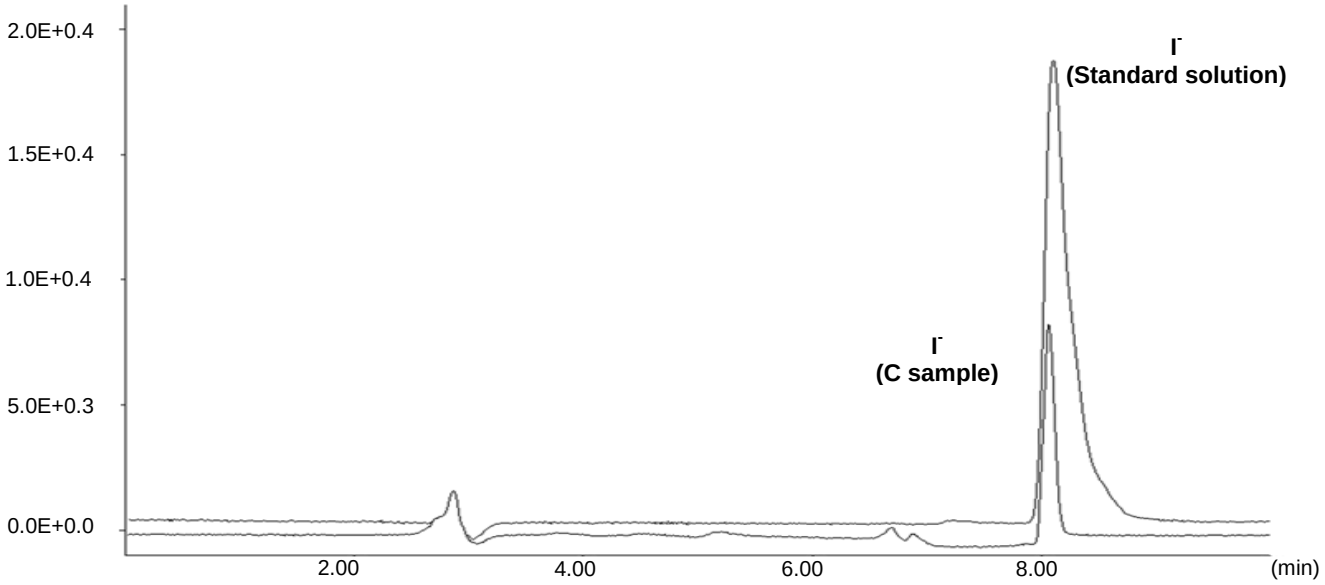

Figure 1 - Representative HPLC chromatograms: Sample C with 1000µg KI/100g flour and standard solution with 5mg L^{-1} KI

However, it was possible to observe that an amount of iodine loss was caused by this digestion. The mean extraction recovery of iodine at the two standard solutions was 77.1% (Table 2). According *Xiaolin Hou et al*[37] the extraction recovery was lower (32.5% for flour) than the obtained for the similar ashing procedure. The average loss of iodine during the sample preparation was 22.9% which may be due to iodine volatilization and/or discarded residue which can include iodine[37]. To improve the extraction more studies are request.

Table 2 – Absolute recovery of iodine from standard solutions (n=4)

Standard Solutions	**Iodine concentration (µg L^{-1})**		**Recovery (%)**
	Added	Found (mean ± SD)	
Control + 1000 µg L^{-1} iodine (n=2)	1000	768.99 ± 12.04	76.9 ± 1.2
Control + 2000 µg L^{-1} iodine (n=2)	2000	1547.71 ± 23.26	77.4 ± 1.2
Mean			**77.1 ± 1.0**

Linearity, Limit of Quantification and Limit of Detection- The calibration curve was used to evaluate the iodine content in real samples. The calibration equation was: y = 194907x + 130.74. The linearity of the calibration curve was verified over the concentration range of 0.2 - 5 mg L^{-1} with a correlation coefficient of 0.999 (Figure 2).

The limit of detection (LOD) and the limit of quantification (LOQ) in the injected solution were found to be 0.008 mg L^{-1} and 0.027 mg L^{-1} respectively, which were sufficient to determinate iodine in the samples. This values were defined as reported by Currie[40].

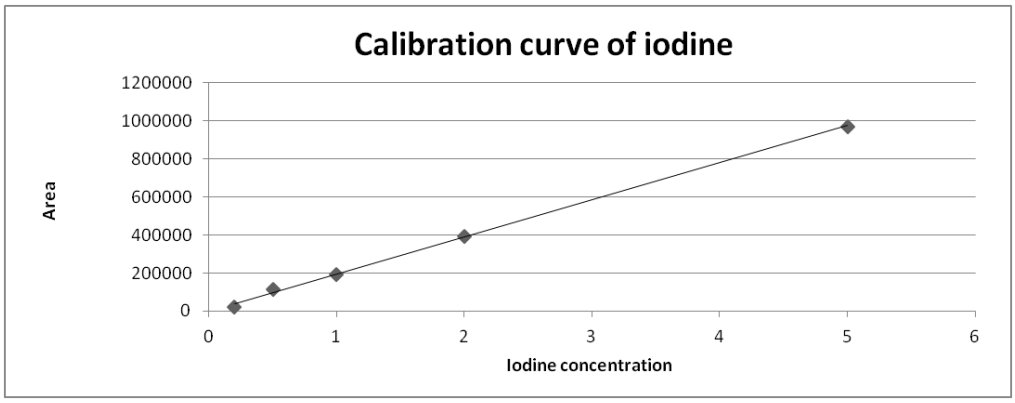

Figure 2 - Calibration curve for various concentrations of iodine

The sweet biscuits iodine concentration was determined approximately one month after being cooked. Although iodine was not added to the control recipe, it was found in this sample. There are several reasons that can explain this: the trace amount of iodine in the different ingredients used, like milk and eggs and some technical inexperience.

The iodine concentration found in the sweet biscuits was substantiality lower than the one added. The iodine concentration found in the several samples was 31.6, 57.6, 79.1 µg/ 100g flour (Figure).

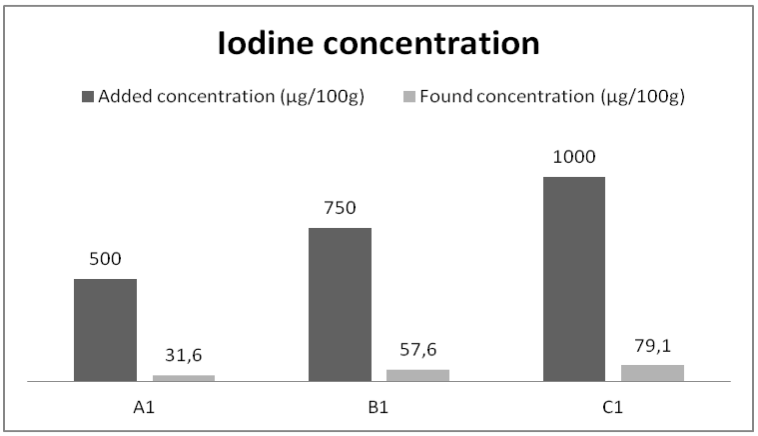

Figure 3 – Results obtained for the iodine content of the sweet biscuits analyzed

The mean recovery of iodine at the three concentrations was 6.3% (A1- low iodine concentration), 7.7% (B1 – medium iodine concentration) and 7.9% (D1- high iodine concentration), respectively (table 3). Besides the losses of iodine during the sample preparation, the conditions of cooking, storage conditions and/or the addition of sugar can cause approximately 69,8% loss of iodine in the processing sweet biscuits[17].

Samples	Moisture (%)	Iodine found (µg/100g)	Recovery (%)
A1	5.82 ± 0.0	31.6 ± 8.1	6.3
B1	5.97 ± 0.0	57.6 ± 16.2	7.7
C1	5.40 ± 0.0	79.1 ± 11.1	7.9

Table 3 – Amount of iodine presented in a dry residue form, found in fortified sweet biscuits

Since elementary iodine and some of its compounds are highly volatile[3], the determination of this mineral in food has been a difficult analytical problem for many years[2]. There are few evidences about iodine stability in the preparation and analysis process. However, *Xiaolin Hou et a l*[37] suggests that the recovery of iodine in the sample preparations analyzed is always lower than 100%. Moreover,

a study[24] suggest a negligible loss of iodine when iodized salt was replaced in the manufacture of processed cereal foods, while other study[17] have shown that the cooking method, kind of cooking utensil or pH have not significantly affected loss of iodine. But the loss became higher with the addition of sugar, food additives, fortificants and spices[17].

Although a diversity of sample pretreatment and analytical iodine quantification methods have been developed, inconsistent results have been obtained. So, it is required to overcome this difficulty: conversion of all iodine species into elementary iodine by distillation and combustion or conversion of all volatile species to nonvolatile ones, such as iodide or iodate[2].

Conclusion

It is technologically possible to do iodine fortified processed cereal food, without compromising the texture, the flavour and the acceptability. The present study shows that there are not only losses of iodine from sample preparation but also from sweet biscuits processing and/or storage conditions.

The limitations of this study are the iodine extraction, the technology of the fortified product and laboratorial inexperience.

The future researches will be interesting analyzed iodine of sweet biscuits after its confection, in order to identify if the loss of iodine are due to processing and/or storage conditions. Moreover, it is essential trying to understand the presence of iodine in the control. Additionally, future studies will focus particularity on the bioavailability of iodine from these sweet biscuits.

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